Role of the Fc Region in the Vitreous Half-Life of Anti-VEGF Drugs

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PURPOSE. To identify the role of the fragment crystallizable (Fc) region in determining intraocular protein drug pharmacokinetics.

METHODS. We generated a new VEGF-Trap lacking the Fc region (Fc∤VEGF-Trap, MWt = 100 kDa) by replacing the Fc region of native VEGF-Trap (MWt = 145 kDa) with a dimerized coiled-coil domain. Forty-two rabbits were injected intravitreally with VEGF-Trap or Fc∤VEGF-Trap ($n = 21$ each) in one of the eyes, harvested at six time points (1 hour and 1, 2, 4, 14, and 30 days after injections). VEGF-Trap and Fc∤VEGF-Trap concentrations in the vitreous, aqueous humor, and retina/choroid were measured, and drug pharmacokinetic properties were analyzed.

RESULTS. In all three ocular compartments, the maximal concentrations for both Fc∤VEGF-Trap and VEGF-Trap were observed at 1 hour after injection. Half-lives of Fc∤VEGF-Trap in the vitreous and retina/choroid (145.02 and 102.12 hours, respectively) were 1.39 and 2.30 times longer than those of VEGF-Trap (103.99 and 44.42 hours, respectively). Total exposure of the aqueous humor and retina/choroid to Fc∤VEGF-Trap was 13.2% and 39% of the vitreous exposure, respectively, whereas VEGF-Trap concentrations were 25.2% and 26.2%, indicating that Fc∤VEGF-Trap shows a preference for posterior distribution and elimination.

CONCLUSIONS. Fc∤VEGF-Trap, despite its lower molecular weight, showed longer half-lives in vitreous and retina/choroid than VEGF-Trap did, suggesting that Fc receptors in ocular tissues contribute to anti-VEGF drug elimination. Truncation or mutation of the Fc region can prolong the intraocular residence time of VEGF-Trap and possibly reduce the number of VEGF-Trap injections required in clinical practice.

Keywords: VEGF-Trap, Fc∤VEGF-Trap, Fc free VEGF-Trap, Fc receptor, Fc region, anti-VEGF, ocular pharmacokinetics

The use of anti-vascular endothelial growth factor (anti-VEGF) agents has revolutionized the treatment of retinal vascular diseases associated with abnormal neovascularization or vascular permeability, including exudative age-related macular degeneration, diabetic macular edema, and macular edema secondary to retinal vein occlusion.[1] Despite the success of potent anti-VEGF agents in treating diverse retinal disorders associated with the overproduction of VEGF, problems such as short half-lives and a high injection frequency remain unresolved.

Three types of anti-VEGF antibodies are currently used for the treatment of age-related macular degeneration and retinal vascular disorders: the Food and Drug Administration–approved drugs ranibizumab and aflibercept, and the off-label drug bevacizumab.[2] The molecular weights of bevacizumab (149 kDa) and ranibizumab (48.39 kDa) are considerably different because ranibizumab does not have a fragment crystallizable (Fc) region and bevacizumab is N-glycosylated in its Fc region.[3]

Aflibercept (VEGF Trap-Eye, Eylea) is not a monoclonal antibody, but a recombinant fusion protein consisting of portions of the human VEGF receptor (VEGFR) 1 and VEGFR2 extracellular domain fused to the Fc region of human IgG1.[4] Generally, drugs with larger molecular weights are thought to
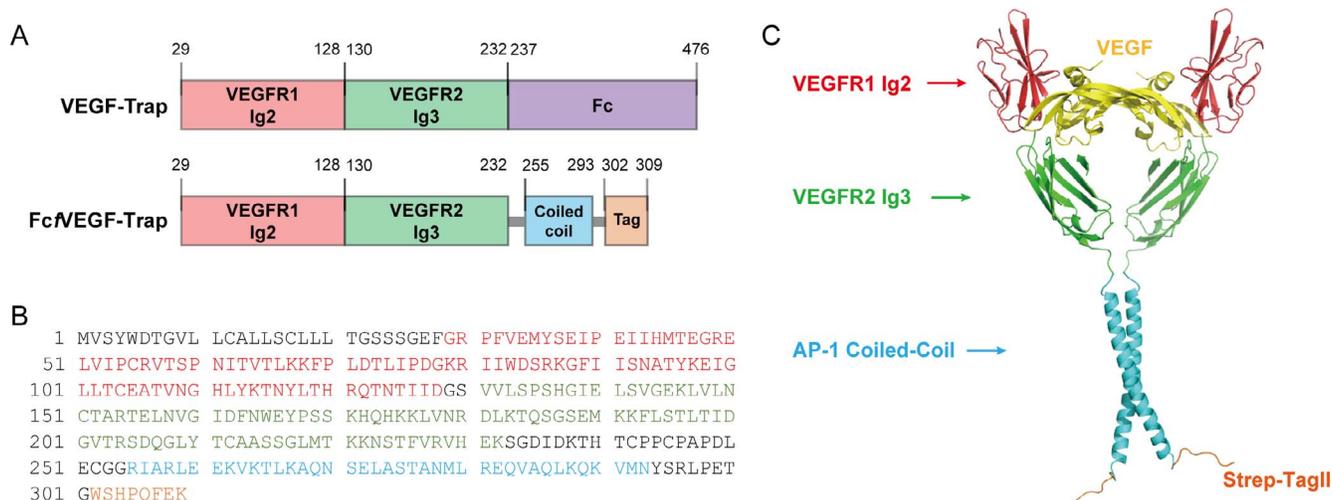
FIGURE 1. Protein structures and amino acid sequences of anti-VEGF analogues: conventional VEGF-Trap and Fc-truncated VEGF proteins (Fc*f*VEGF-Trap). (**A**) Fc*f*VEGF-Trap contains a dimerized coiled-coil domain (AP-1, UniProtKB ID: P05412, 276R~314N) instead of the Fc domain in VEGF-Trap, which is a fusion protein containing human VEGFR1-Ig2 and VEGFR2-Ig3 and the human Fc domain. (**B**) Fc*f*VEGF-Trap is composed of 309 amino acids, and the predicted molecular weight of Fc*f*VEGF-Trap is two-thirds of that of VEGF-Trap. (**C**) The model structure of Fc*f*VEGF-Trap/VEGF-A complex.

have prolonged vitreous half-life.[5] In fact, it was previously reported that the vitreous half-life of ranibizumab (2.75 days) was shorter than those of bevacizumab (7.06 days) and aflibercept (3.63 days) in rabbits.[6-9]

Intravitreally administered anti-VEGF drugs reach first and in a larger proportion in the external layer (pericytes) and then reach the inner layer (endothelial cells) after crossing the blood–retina barrier.[10] Recent studies reported that the elimination of intravitreally administered IgG across the blood–retina barrier into the systemic circulation is mediated by the neonatal Fc receptor (FcRn), which is expressed in the retinal pigment epithelium and endothelial cells of the retinal and choroidal vasculature.[11-13] Ocular application of the Fc-based fusion proteins is a relatively new advance, and whether their elimination or transport depends on FcRn and/or the molecular weight remains unclear. Understanding of the role of the Fc region and FcRn in intraocular pharmacokinetics (PK) is important in determining the duration of therapeutic efficacy of drugs as well as in anticipating the systemic exposure of intravitreally injected anti-VEGF agents, which can potentially cause systemic complications.

During the past few years, we have reported the intraocular PK of bevacizumab, ranibizumab, VEGF-Trap, and aflibercept, which are representative anti-VEGF agents currently used in the clinic.[6-9] Based on our experience and skills in ocular PK, we comparatively studied the ocular PK of VEGF-Trap and a newly synthesized Fc region–free VEGF-Trap (Fc*f*VEGF-Trap) to uncover the role of Fc region in ocular PK.

MATERIALS AND METHODS

Generation of Fc*f*VEGF-Trap, a VEGF-Trap–Based Protein Obtained by Replacing the Fc Region of Human IgG1 With a Dimerized Coiled-Coil Domain

Previously, we generated VEGF-Trap, a fusion protein containing human VEGFR-Ig2 (UniProt ID: P17948), VEGFR-Ig3 (UniProt ID: P35968), and the human Fc domain, based on the method reported earlier.[8] The generated VEGF-Trap consisted of 476 amino acids with a sequence similarity of approximately 95% to commercially available VEGF-Trap-Eye,

aflibercept (Eylea; Regeneron, Inc., and Bayer Healthcare Pharmaceuticals, Berlin, Germany), which consisted of 458 amino acids. We reported the results of ocular PK of VEGF-Trap in our previous study.[8] Fc*f*VEGF-Trap was generated based on VEGF-Trap sequences. Instead of the Fc domain of VEGF-Trap, Fc*f*VEGF-Trap contains a dimerized coiled-coil domain derived from transcription factor AP-1 (UniProtKB ID: P05412, 276R-314N) followed by Strep-Tag II (8 amino acid sequence, WSHPQFEK) at the C-terminus. Stable cell lines expressing Fc*f*VEGF-Trap were generated from dihydrofolate reductase (DHFR)-deficient Chinese hamster ovary cells selected using G418 and methotrexate. Secreted Fc*f*VEGF-Trap was purified from culture media using Strep-Tactin resin (IBA, Göttingen, Germany) according to the manufacturer's instructions.[14] Briefly, Strep-Tactin–bound Fc*f*VEGF-Trap was eluted in a competitive manner using desthiobiotin. To remove the remaining desthiobiotin, eluted protein was dialyzed with PBS overnight. Proteins were quantified by Bradford assay, and protein purity was confirmed by SDS-PAGE and Coomassie Blue staining.

While conventional VEGF-Trap is composed of two VEGFR-Igs fused to an Fc domain, Fc*f*VEGF-Trap includes a dimerized coiled-coil domain instead of the Fc domain (Figs. 1A, 1B). The predicted molecular weight of Fc*f*VEGF-Trap is approximately 100 kDa, two-thirds that of VEGF-Trap (145 kDa).[8] The model structure of Fc*f*VEGF-Trap/VEGF-A complex was generated using VEGFR1 D2 structure (protein data bank [PDB]: 5ABD), VEGFR2 D2-D3/VEGF-A complex structure (PDB: 3V2A), and AP-1 coiled-coil structure (PDB: 1JUN) (Fig. 1C). This coiled-coil domain and Strep-Tag II composed of 55 amino acids can stabilize the dimeric Fc*f*VEGF-Trap without the Fc region and decrease the molecular weight of VEGF-Trap without changing the amino acid sequence of the active region responsible for targeting the VEGF receptor–binding site.

Animal Studies

This study was approved by the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee, and all experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

A total of 42 eyes from 42 New Zealand White rabbits weighing approximately 1.5 to 2.0 kg were randomly assigned to VEGF-Trap ($n = 21$) or Fc/VEGF-Trap ($n = 21$) groups. The intraocular PK of VEGF-Trap and Fc/VEGF-Trap were evaluated using same experimental design as in our previous studies.[6–9] Intramuscular injection of 15 mg/kg Zoletil (a mixture of tiletamine and zolazepam; Virbac Laboratories, Carros, France) and 5 mg/kg xylazine, and a topical ophthalmic anesthetic (1% proparacaine hydrochloride, Alcaine; Alcon Laboratories, Inc., Fort Worth, TX, USA) were used for anesthesia. Eyes under investigation were dilated with eye drops containing a mixture of phenylephrine and tropicamide. Povidone iodine solution (5%) was applied for periocular skin and conjunctival antisepsis. Next, all intravitreal injections of VEGF-Trap (0.3 mg/0.03 mL) or Fc/VEGF-Trap (0.2 mg/0.03 mL) were performed in the right eye. To administer the same molar dose of Fc/VEGF-Trap and VEGF-Trap, the dose of Fc/VEGF-Trap was matched to two-thirds of the dose of VEGF-Trap because the molecular weight of Fc/VEGF-Trap is two-thirds that of VEGF-Trap. A sterile 30-gauge needle was introduced 1 mm posterior to the surgical limbus in the superotemporal quadrant of the ocular globe. Three or four rabbits were killed at each of the following time points: 1, 24, 48, 120, 336, and 720 hours (1 hour and 1, 2, 5, 14, and 30 days) after injection. Enucleated eyes were immediately stored at −80°C until analysis. Before immunoassay, eyes were divided into vitreous, aqueous humor, and retina/choroid compartments. To solubilize the vitreous, samples were soaked in PBS with 1% BSA at 4°C overnight and centrifuged at 387g for 10 minutes. Tissue proteins were extracted from the lysates of homogenized retina/choroids using lysis reagent (CellLytic MT, C3228; Sigma-Aldrich Corp., St. Louis, MO, USA) and a homogenizer.

Drug Immunoassay

We measured the concentration of Fc/VEGF-Trap in each compartment at serial time points by an indirect enzyme-linked immunosorbent assay (ELISA) in accordance with previous reports.[8,15] The diluted 165 amino acid antigen solution of human recombinant VEGF (rVEGF) (10 μg/mL) in a 50 mM carbonate buffer (pH 9) was divided into aliquots in 96-well plates at 100 μL/well. The plates were incubated with rVEGF antigens at 4°C overnight, washed with PBS, blocked with PBS containing 1% BSA at 4°C for 4 hours, and dried at 4°C in an incubator. Vitreous, aqueous humor, and retina/choroid samples were diluted in PBS with 0.1% BSA, put into a 96-well plate, and incubated at 4°C overnight to form antigen and antibody (VEGF-Trap or Fc/VEGF-Trap) complexes. We incubated Strep-Tactin conjugated with horseradish peroxidase (HRP) (1:5000; Bio-Rad, Hercules, CA, USA, no. 1610381) to detect rVEGF-Fc/VEGF-Trap complex, and 1:20,000 goat anti-human IgG/Fc antibody labeled with HRP (Abcam, Cambridge, MA, USA) to detect rVEGF-VEGF-Trap, in 96-well plates for 2 hours at room temperature. 3,3′,5,5′-Tetramethyl benzidine substrate was used to detect HRP activity, and the optical density of the color change, which reflects the VEGF-Trap or Fc/VEGF-Trap concentration, was determined using a standard ELISA plate reader (Bio-Rad) and embedded software, SoftMax Pro 5.4.1 (Molecular Devices, Sunnyvale, CA, USA). Using standard curves, the concentrations of VEGF-Trap and Fc/VEGF-Trap in our samples were calculated. The drug concentration in retina/choroid was defined as the weight of drugs (mg) to the weight of the retina/choroid tissue (g).

Pharmacokinetic Data Analysis

Quantitative analysis of all samples was performed using a four-parameter logistic (4PL) curve, which is widely used in curve-fitting analysis for typical ELISAs, and is more reasonable for describing biological systems than linear curve or semilog plot.[16] The changes in concentrations of VEGF-Trap and Fc/VEGF-Trap in the vitreous, aqueous humor, and retina/choroids were analyzed by one- and two-compartment models. The equations and detailed parameters used for pharmacokinetic modeling and analysis were described previously.[8,17] The half-life of elimination ($t_{1/2}$), mean residence time (MRT), observed maximum concentration (C_{max}), area under the concentration–time curve (AUC), apparent volume of distribution (V_d/F), and apparent clearance (CL/F) were estimated. Using the AUC for each compartment, the total exposure of the aqueous humor and retina/choroid to VEGF-Trap and Fc/VEGF-Trap from the vitreous was calculated. Statistically, the mean values and standard deviation of drug concentration at each time point were calculated, and the estimated curves were plotted using these values. Estimated pharmacokinetic data are presented as parameter estimate (CV%) values. A low CV value indicates reliable parameter without large variability.

RESULTS

No adverse events or signs of ocular inflammation were observed after intravitreal injection of either VEGF-Trap or Fc/VEGF-Trap. The changes in estimated amounts and concentrations over time for VEGF-Trap and Fc/VEGF-Trap in the vitreous, aqueous humor, and retina/choroid samples are shown in Table 1. The estimated concentration–time curves with observed concentrations at the six time points for VEGF-Trap and Fc/VEGF-Trap are shown in Figure 2. The concentrations of VEGF-Trap and Fc/VEGF-Trap in the aqueous humor and retina/choroids, as well as the vitreous, declined in a biexponential fashion. For the vitreous, one-compartment model could explain the PK of FcfVEGF-Trap and VEGF-Trap, while data fitting could not be achieved in other models (Fig. 3). For the aqueous humor and retina/choroids, the two-compartment model was selected, considering physiological compartment as well as AIC and CV values. For the two-compartment model, Akaike's information criterion (AIC) values of Fc/VEGF-Trap in the aqueous humor and retina/choroids were 20.42 and 12.54, and those of VEGF-Trap were 19.72 and 32.55, respectively. The C_{max} of VEGR-Trap and Fc/VEGF-Trap in the vitreous were 67.37 and 37.44 μg/mL at 1 hour after injection of equal molar dose of Fc/VEGF-Trap (0.2 mg/0.03 mL) and VEGF-Trap (0.3 mg/0.03 mL). Similarly, the C_{max} of both drugs in the aqueous humor and retina/choroid was reached at 1 hour (Table 2). The estimated half-lives of Fc/VEGF-Trap in the vitreous and retina/choroid were 1.39 and 2.30 times longer (145.02 and 102.12 hours, respectively) than those of VEGF-Trap (103.99 and 44.42 hours, respectively). The MRT of Fc/VEGF-Trap and VEGF-Trap was 209.22 and 150.02 hours, respectively. Likewise, the dose-normalized AUC of Fc/VEGF-Trap in the vitreous was 1.162 times higher than that of VEGF-Trap. In addition, the total exposure of the aqueous humor and retina/choroid to Fc/VEGF-Trap was approximately 13.2% and 39% that of the vitreous exposure, respectively, whereas VEGF-Trap concentrations in the aqueous humor and retina/choroid were approximately 25.2% and 26.2% that of the vitreal exposure, respectively. These results indicate that the anterior excretion of Fc/VEGF-Trap is relatively low and the posterior excretion is relatively high, compared to that of VEGF-Trap, suggesting that Fc/VEGF-Trap shows a preference for posterior excretion. The V_d/F values of Fc/VEGF-Trap were higher than those of VEGF-Trap in the vitreous (5.34 vs. 4.45 mL) and the retina/choroid (22.84 vs. 15.25 mL), but not in the aqueous humor (22.26 vs. 33.62 mL). These results indicate that Fc/VEGF-Trap is mainly distributed in the posterior

TABLE 1. The Concentrations and Amounts of VEGF-Trap and Fc/VEGF-Trap in the Vitreous, Aqueous Humor, and Retina/Choroid of Rabbit Eyes at 1 Hour and 1, 2, 5, 14, and 30 Days Post Injection

| | Fc/VEGF-Trap | | | | | | VEGF-Trap | | | | | |
| | Vitreous | | Aqueous Humor | | Retina/Choroid | | Vitreous | | Aqueous Humor | | Retina/Choroid | |
Time	Conc, µg/mL	Amount, µg	Conc, µg/mL	Amount, µg	Conc, µg/g	Amount, µg	Conc, µg/mL	Amount, µg	Conc, µg/mL	Amount, µg	Conc, µg/g	Amount, µg
1 h	34.43 ± 14.54	51.65 ± 14.65	16.06 ± 0.61	3.21 ± 0.12	13.60 ± 1.58	0.33 ± 0.04	73.09 ± 9.63	109.63 ± 14.45	59.91 ± 15.02	11.98 ± 3.00	21.70 ± 7.24	0.52 ± 0.17
1 d	32.59 ± 4.08	48.89 ± 6.13	11.63 ± 2.25	2.33 ± 0.45	10.99 ± 0.18	0.26 ± 0.00	50.62 ± 4.78	75.93 ± 7.17	33.46 ± 1.42	6.69 ± 0.28	17.24 ± 2.91	0.41 ± 0.07
2 d	32.57 ± 0.46	48.85 ± 0.69	4.58 ± 0.60	0.92 ± 0.12	6.73 ± 1.65	0.16 ± 0.04	43.87 ± 1.08	65.81 ± 1.61	12.30 ± 1.41	2.46 ± 0.28	9.54 ± 4.74	0.23 ± 0.11
5 d	25.39 ± 3.01	38.08 ± 4.52	2.61 ± 0.92	0.52 ± 0.18	5.02 ± 1.62	0.12 ± 0.04	38.70 ± 5.61	58.04 ± 8.41	8.41 ± 0.17	1.68 ± 0.03	5.08 ± 2.23	0.12 ± 0.05
14 d	1.80 ± 0.12	2.70 ± 0.18	0.11 ± 0.19	0.02 ± 0.04	1.66 ± 1.48	0.04 ± 0.04	2.94 ± 0.16	4.41 ± 0.24	2.02 ± 0.20	0.40 ± 0.04	–	–
30 d	1.51 ± 0.17	2.26 ± 0.25	0.31 ± 0.15	0.06 ± 0.03	0.61 ± 0.62	0.01 ± 0.01	1.42 ± 0.51	2.12 ± 0.77	0.73 ± 0.02	0.15 ± 0.00	–	–

The volumes of the vitreous and aqueous humor of the rabbit were considered 1.5 and 0.2 mL, respectively, and the weight of the retina/choroid was considered 0.024 g. Data are means ± SD. Conc, concentration; amount, total amount in the compartment.

segment of the eyeball, whereas a large portion of intravitreally injected VEGF-Trap is distributed in the anterior segment.

DISCUSSION

In this study, we investigated and analyzed the ocular PK of an Fc region–free VEGF-Trap and compared it with that of VEGF-Trap. The lower molecular weight of the Fc/VEGF-Trap (compared to native VEGF-Trap) may promote initial elimination from the vitreous. However, the replacement of the Fc region with a dimerized coiled-coil domain may enhance the long-term intraocular retention of Fc/VEGF-Trap, which was found to be approximately 40% longer than that of the conventional VEGF-Trap in this study.

According to previous reports, molecular weight is one of the determinant factors for ocular PK. The rate of diffusion is approximately inversely proportional to the cube root of the molecular weight; therefore, high molecular weights are thought to prolong vitreous half-life.[18] However, in our study, the low molecular weight Fc/VEGF-Trap showed a 1.39 times longer vitreous half-life than VEGF-Trap (145.02 vs. 103.99 hours) did. This suggests that the elimination of vitreous VEGF-Trap is not merely through a molecular weight–dependent mechanism and that Fc region is associated with the prolongation of intraocular half-life. Considering the smaller molecular weight of Fc/VEGF-Trap (100 kDa) compared to VEGF-Trap (145 kDa), the actual amount of FcRn-dependent elimination of VEGF-Trap is estimated to be larger than our result.

The elimination of an intravitreally injected drug is achieved through two main routes: anterior and posterior. Anterior elimination refers to the diffusion of vitreous drugs into the posterior chamber and turnover via aqueous and uveal flow. Posterior elimination is achieved through permeation across the posterior blood–eye barrier.[5] The initial concentration (1 hour after intravitreal administration) of Fc/VEGF-Trap was relatively lower than that of VEGF-Trap in all three compartments, even allowing for the dosage difference between the two drugs to match the molar dose (Fig. 2). There was a sharp decrease in Fc/VEGF-Trap levels, which was more than that for VEGF-Trap, in the early time period. Although the exact mechanism for this sharp decrease could not be identified, it might be associated with the low molecular weight of Fc/VEGF-Trap. The low molecular weight Fc/VEGF-Trap could achieve early dynamic equilibrium, or a large amount of drug could be transported into the anterior chamber.[19,20] After the rapid decline phase of drug concentration, a second phase was observed in which the relative concentration of Fc/VEGF-Trap was lower in the aqueous humor and higher in the retina/choroid than that of VEGF-Trap, suggesting that Fc/VEGF-Trap shows a preference for posterior excretion. Furthermore, the apparent V_d/F of Fc/VEGF-Trap, which may be correlated with the amount of drug distributed into the tissue, was significantly higher in the posterior compartment and lower in the anterior compartment, compared to that of VEGF-Trap. This indicates that Fc/VEGF-Trap is more readily distributed in the posterior segment of the eyeball than VEGF-Trap, whereas a larger portion of intravitreally injected VEGF-Trap is distributed in the anterior segment than is Fc/VEGF-Trap. This higher preference for second-phase distribution of Fc/VEGF-Trap in the posterior compartment may be associated with the replacement of the Fc region with the coiled-coil domain. Our previous study illustrated that the AUC of ranibizumab in aqueous humor and retina/choroid were 3.72% and 20.46% that of vitreous, respectively, suggesting that a monoclonal antibody fragment (Fab) lacking an Fc region had similar preference for posterior distribution. Eventually, the preference for posterior elimina-
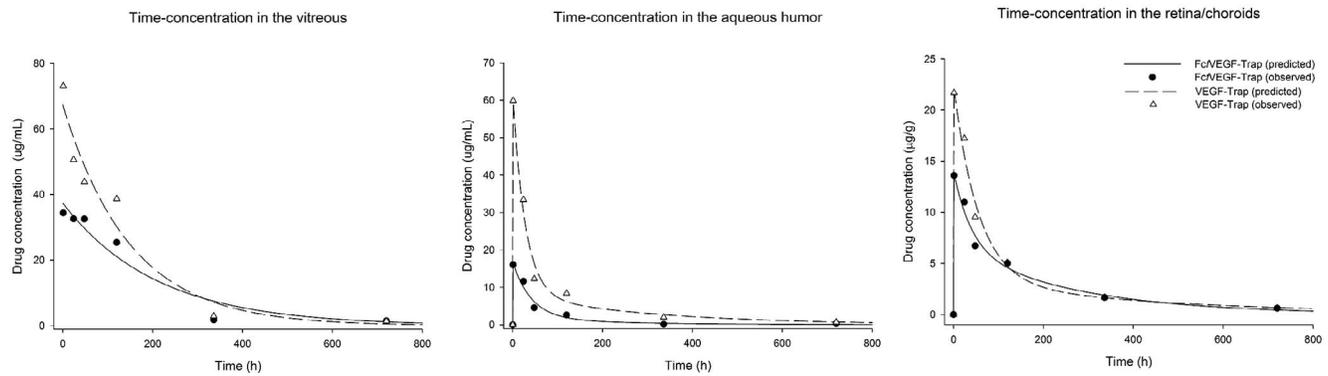
FIGURE 2. Concentration of Fc*f*VEGF-Trap and VEGF-Trap in the rabbit eyes. *Points* represent observed concentrations and *lines* represent estimated concentrations by models.

tion possibly enhances the efficacy of Fc*f*VEGF-Trap by increasing the delivery of anti-VEGF molecules into the retina.

The common anti-VEGF agents, including bevacizumab and aflibercept, are Fc-containing proteins. The main receptors for these proteins are the Fcγ receptors (FcγR) on cell membrane and FcRn. The expression of FcγR has been previously demonstrated using an RPE model: the ARPE-19 cell line. FcRn is vital for increasing the serum circulating half-life of antibodies and for protecting them from lysosomal degradation.[12] Hence, it is an important factor in systemic PK of antibodies. However, the function of FcRn in intraocular PK was not reported until recently, when studies showed the presence of FcRn on RPE cells; moreover, recent studies showed that FcRn, which is involved in intracellular uptake and transport of Fc-containing molecules in the retina, played an essential role in eliminating intravitreally administered IgGs across the blood–retina barrier into the systemic circulation.[13,21] Despite recent studies regarding FcRn, the function of the Fc region in ocular PK remains controversial. Some reports showed that inhibition of FcRn increased apical to choroidal transport of bevacizumab, indicating a role of FcRn in the recycling of the molecule.[12] Another study showed that protein molecular weight and Fc region did not play critical roles in ocular PK as they do systemically.[22] According to our results, VEGF-Trap showed Fc region–dependent properties in ocular PK. Therefore, we suggest that the Fc region of VEGF-Trap may play a role in vitreoretinal-specific elimination of VEGF-Trap and that the Fc region is a potential factor that diminishes VEGF-Trap concentration in the posterior compartment of the eyeball. By considering these characteristics of VEGF-Trap associated with Fc region–dependent clearance, we can advance our understanding of ocular PK of anti-VEGF agents.

We generated a new Fc*f*VEGF-Trap by replacing for the Fc domain of VEGF-Trap with AP-1 coiled-coil domain. The coiled-coil structure has been a primary target of protein engineering for its potential applications such as drug delivery.[23] The coiled-coil structure of AP-1 induces heterodimerization of the hydrophobic residues on c-Fos and c-Jun.[24] This AP-1 coiled coil can also generate a stable heterodimer of VEGFR1 D2-VEGFR2 D3, similar to the Fc region of VEGF-Trap. AP-1 is a well-known transcription factor that regulates a number of cellular processes, including cell growth, differentiation, and apoptosis by binding to specific DNA sequences.[25] Thus, we believe that there is little chance that the coiled-coil domain of specific transcription factor will nonspecifically bind to other proteins. Furthermore, in a recent study, Deacon et al.[26] investigated the ability of a polymer to form a stable coiled-coil heterodimer with the target c-Jun sequence of the oncogenic AP-1 transcription factor using two-2D ^{15}N-HSQC NMR and a recombinant ^{15}N-labeled c-Jun peptide ([^{15}N]r-c-Jun). The heterodimerization was successful and, importantly, the polymer did not sterically disadvantage hybridization. Based on their findings, the authors suggested an important role of polymer-coiled-coil peptide conjugates in future drug delivery.[26] Similarly, a recombinant enzyme containing a c-terminal coiled-coil peptide was reported to have strong activity and high thermochemical stability compared with the wild-type enzyme.[27] Moreover, a helix-stabilized antibody fragment (hsFv) obtained by fusing a coiled coil to the Fv fragment of an antibody possessed similar expression, stability, and oligomerization properties to other Fv constructs.[28] This substitution may protect Fc*f*VEGF-Trap from Fc region–related elimination in addition to stabilizing the Fc*f*VEGF-Trap structure, and may eventually contribute to longer residence time. Clinically, the longer residence time of Fc*f*VEGF-Trap has the advantage of reducing the frequency of intravitreal injection and the chances of systemic side effects, such as ischemic heart disease or cerebrovascular disorders.[29]

TABLE 2. Estimated Pharmacokinetic Parameters of Fc*f*VEGF-Trap and VEGF-Trap After Intravitreal Injection Into Rabbit Eyes

Drug	Compartment	K_e, Terminal (h^{-1})	$t_{1/2}$ (h)	MRT (h)	C_{max} (µg/mL)	AUC (h*µg/mL)	V_d/F (mL)	CL/F (mL/h)
Fc*f* VEGF-Trap, 200 µg	Vitreous humor	0.0048 (26.11)	145.02 (26.09)	209.22 (26.09)	37.44 (8.05)	7832.22 (22.46)	5.34 (8.06)	0.026 (22.48)
	Aqueous humor	0.0161 (76.24)	43.02 (76.17)	114.92 (328.86)	16.46 (8.38)	1032.68 (75.50)	22.26 (259.95)	0.194 (75.58)
	Retina/choroids	0.0068 (26.86)	102.12 (26.83)	234.40 (53.63)	13.83 (6.00)	2052.42 (25.97)	22.84 (32.02)	0.097 (25.99)
VEGF Trap, 300 µg	Vitreous humor	0.0067 (25.67)	103.99 (25.64)	150.02 (25.64)	67.37 (8.36)	10107.73 (21.69)	4.45 (8.36)	0.029 (21.71)
	Aqueous humor	0.0088 (57.55)	78.89 (57.50)	285.36 (183.96)	22.20 (5.79)	2546.34 (56.70)	33.62 (132.37)	0.118 (56.76)
	Retina/choroids	0.0156 (58.11)	44.42 (58.06)	200.47 (161.33)	60.58 (5.47)	3944.30 (57.70)	15.25 (109.07)	0.076 (57.75)

Data are presented as parameter estimate (CV%). Ke, elimination rate constant; $t_{1/2}$, half-life; MRT, mean residence time; C_{max}, observed maximum concentration; AUC, area under concentration-time curve; V_d/F, apparent volume of distribution; CL/F, apparent clearance.
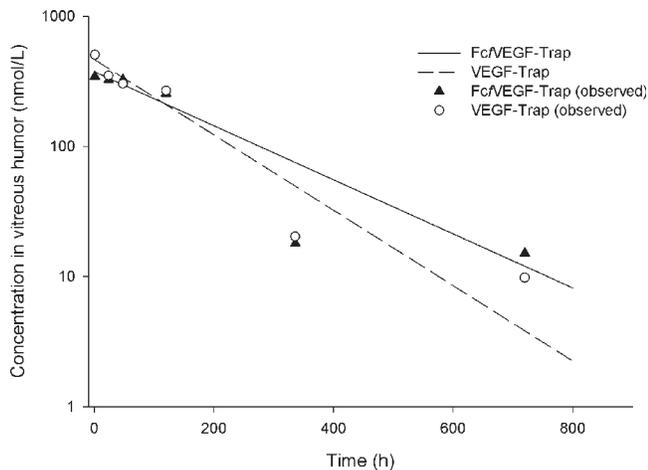
FIGURE 3. Vitreous concentrations of study drugs in the rabbit eye. This graph shows first-order eliminations of Fc*f*VEGF-Trap and VEGF-Trap, estimated from one-compartment models. Difference of slopes of the two plots implies that vitreous half-life of Fc*f*VEGF-Trap is longer than that of VEGF-Trap.

One of the limitations of our study is that the molecular weight of Fc*f*VEGF-Trap is lower than that of VEGF-Trap, and this may affect ocular PK, although these two drugs are predicted to have similar chemical properties. However, the longer half-life of Fc*f*VEGF-Trap despite its lower molecular weight emphasizes the role of Fc region in ocular PK. We replaced the Fc region with the coiled-coil domain, which was presumed not to affect the chemical properties of VEGF-Trap; but the coiled-coil domain itself might affect ocular PK by changing the chemical properties of the drug. Furthermore, we neither investigated the stability of Fc*f*VEGF-Trap nor performed a functional comparison of Fc*f*VEGF-Trap and VEGF-Trap. Despite these limitations, our study is the first to investigate the effect of Fc region on ocular PK by generating a novel molecule of Fc region–free VEGF-Trap.

In conclusion, Fc-region–deficient VEGF-Trap showed significantly longer vitreous and retina/choroid half-lives than conventional VEGF-Trap despite its lower molecular weight, indicating that Fc receptors in ocular tissues contribute to drug elimination. Our findings may be useful in future development of new anti-VEGF agents for intraocular administration. Truncation or mutation of the Fc region of protein drugs can prolong the intraocular residence time, reducing the number of injections and the systemic exposure to intraocular drugs.

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